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Phenotypic Differences in Populations of Euonymus Americanus L. from the Central United States & Autecological Studies of this Species in a Deciduous Forest of Kentucky

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PHENOTYPIC DIFFERENCES IN POPULATIONS OF *EUONYMUS AMERICANUS* L. FROM THE CENTRAL UNITED STATES AND AUTECOLOGICAL STUDIES OF THIS SPECIES IN A DECIDUOUS FOREST OF KENTUCKY

A Thesis

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In Partial Fulfillment

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Master of Science

by

Paul Eric Bayer

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PHENOTYPIC DIFFERENCES IN POPULATIONS OF _EUONYMUS AMERICANUS_ L. FROM THE CENTRAL UNITED STATES AND AUTECOLOGICAL STUDIES OF THIS SPECIES IN A DECIDUOUS FOREST OF KENTUCKY

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Dedicated to my mother, who took an active interest in my work by making her own observations on this plant and then suggesting new avenues of research to pursue, and to my father, who encouraged me and removed some of the financial burdens from my shoulders.

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I am sure that I have inadvertently forgotten to mention several individuals, and I would like to acknowledge their assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PHENOTYPIC DIFFERENCES IN POPULATIONS OF EUONYMUS AMERICANUS L. FROM THE CENTRAL UNITED STATES AND AUTECOLOGICAL STUDIES OF THIS SPECIES IN A DECIDUOUS FOREST OF KENTUCKY

Paul Eric Bayer May, 1984 49 pages

Directed by: J.E. Winstead, G.E. Dillard and F.R. Toman

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The concentrations of sodium and potassium in the leaves of Euonymus americanus collected from a relict hardwood forest during a four month period showed a possible winter conditioning pattern. Seeds collected from this same relict stand would not germinate, even though they were subjected to a wide variety of germination experiments. Phenotypic differentiation in fully developed leaves was not found; however, differences related to the length of the growing season were noted. Autecological studies of Euonymus americanus should be continued to investigate the role of this plant in relict hardwood stands.

INTRODUCTION

The presence of characteristic vegetation in certain regions of the United States during specific stages of successional development has long been noted (Braun-Blanquet, 1932; Clements, 1928). In south central Kentucky, the climax vegetation type was probably an oak-hickory hardwood forest (Küchler, 1964). Today however, only isolated patches of this hardwood forest remain (Bougher and Winstead, 1974). Euonymus americanus L. is a very common understory shrub in these remnant hardwood forests with a reported distributional frequency exceeding 98% (Bougher and Winstead, 1974). The reason for its presence in such abundance is unknown. The only reported studies on this plant involve its chromosome number (Nath and Clay, 1972) and its importance as a food source for deer, bluebirds and robins (Halls and Alcaniz, 1972: Blakelock, 1951). The great majority of research on this genus has focused on seed germination and embryo development in European species (Alekseeva, 1975a, 1975b; Beranger-Novat and Monin, 1971a, 1971b; Beranger-Novat et al., 1977; Gasperi-Campani et al., 1980; Lyubchenko, 1959; Monin, 1965, 1967a, 1967b, 1967c, 1967d, 1968; Naumova, 1970a, 1970b; Nikolaeva, 1959; Nikolaeva et al., 1973; Nikolaeva et al., 1974). Chromosome numbers in Euonymus have been investigated by Nath and Clay (1972), Sareen et al. (1974) and Skalinska et al. (1976). One additionally important study on nomenclature reveals that the correct spelling is *Euonymus* and not *Evonymus* (Vaczy, 1975).

Studies relating to successional patterns of *Euonymus* spp., and specifically *E. americanus*, have not been conducted. Ecotypic differentiation in this genus has been similarly overlooked. In an effort to determine whether the observed high frequency of *E. americanus* in a relict climax hardwood forest was related to sexual reproduction, seed germination experiments were conducted. The extent of sodium and potassium cycling in these relict stands was also investigated to determine whether a "pumping" of either ion exists similar to that reported for calcium (Thomas, 1969). An additional investigation of geographic variation in the leaves of *E. americanus* was related to potential ecotypic differentiation.

Euonymus americanus is a member of the Celastraceae or Stafftree family (Strausbaugh and Core, 1973) and is only one of over 120 species of *Euonymus* distributed worldwide. Most of these species are cultivated, but *E. americanus* is wild and found exclusively in the United States. The distribution of this plant can be delimited by an imaginary line extending west from southern New York to southern Illinois and then southwest to eastern Texas. The entire region east and south of this line with the exception of southern Florida contains *E. americanus* (Bailey, 1949).

Euonymus americanus looks very much like *E. obovatus* Nutt. but is distinguishable because the stem is upright rather than procumbent, and the leaves are lanceolate rather than obovate (Bailey, 1949). Flowers are produced in May, and each gives rise to a loculicidal capsule in late September (Jones and Luchsinger, 1979). The capsules look like strawberries, give the plant its common name (Strawberry Bush or Bursting Heart) and are the only means of sexual reproduction. Within the 3-5 lobed capsule are arils containing the seeds. The aril is a bright orange color and usually contains 3-5 seeds. Birds may eat the arils and eventually deposit seeds in new locations (Blakelock, 1951). Although seeds provide the only method of sexual reproduction, *E. americanus* reproduces primarily by asexual means. Runners are produced from the base of the stem and grow horizontally along the substrate. These runners often develop roots and sometimes produce a new stem. Eventually the interconnecting runners will be severed and separate plants will be formed. The newly formed plants have the same genotype as the parent plant being clones of the parent. Clonally produced plants are often quite successful. Other common plant clones include Huckleberry, Quaking aspen, Black spruce and Starflower (Cook, 1983). Although asexual reproduction occurs primarily in the above manner, a preliminary investigation of stem cuttings revealed that 95% of the stems could be rooted by placing them in water, indicating possible dispersal by cuttings.

The survival of this plant through asexual means may be an important adaptation to "problems" in sexual reproduction.

Although the haploid number is 32, other species may have haploid numbers of 8, 16, 24 or 32 (Nath and Clay, 1972; Sareen et al., 1974; Skalinska et al., 1976). During meiosis in _E. americanus_, univalents, bivalents, quadrivalents and other abnormalities have been observed (Nath and Clay, 1972). In addition, two distinct sizes of pollen are present indicating that this plant is a tetraploid (Nath and Clay, 1972).

In a genus of this size, there is a great potential for further investigation. The importance of this initial work lies in the fact that data relating to succession and ecotypic differentiation in this genus are lacking. Baseline data for _E. americanus_ on these two important topics would be useful in other studies of this genus. This project was an attempt to investigate the importance of seed germination in maintaining a high frequency of occurrence, and the extent of sodium and potassium cycling in the leaves of this plant, in relict hardwood forests. The degree of ecotypic differentiation in this species was also investigated through an examination of phenotypic differences in the leaves.

METHODS AND MATERIALS

SEED GERMINATION EXPERIMENTS

Capsules containing seeds of *Euonymus americanus* were collected in early October of 1982 and 1983 from a single site in south central Kentucky. The site of collection occurs 16 km east of Interstate 65 on Rte. 68/80 in Barren County and is commonly known as Bonayer Forest.

Capsules and seeds from the 1982 collection were allowed to dry at room temperature for at least 3 months. The seeds were separated from the capsules and the aril removed. The seeds were then coated with semasan, an antifungal agent. Ten seeds were placed in a sterilized petri dish on a single nine centimeter Whatman #1 filter paper disc. The seeds were kept moist with deionized water.

An Environator Corporation Model 3448 growth chamber was used to provide the conditions for seed germination. The chamber was programmed for a continuously cycling 12 h "day" temperature of 29.4 C and a 12 h "night" temperature of 21.1 C with constant fluorescent and incandescent lighting. Several experiments involved a stratification period. A Forma Scientific Model IC 48406 Walk-In Environmental Room was used to provide a constant stratification temperature of 2 C. Seeds placed in this chamber were kept moist but in continuous darkness.

A number of different germination experiments were then conducted with these seeds as noted in Tables 1 and 2. All petri plates reported in Table 1 contained seeds which had been scarified by puncturing the seed coat with a dissecting needle. All seeds reported in Table 2 remained unscarified. All experiments were conducted in duplicate.

Seeds placed in the environmental chamber were either kept in the dark by putting them in black cloth sacks or they were exposed to continuous light. Several experiments involved placing the seeds in a 3% HCl/H_2O solution for 24 h. The seeds were removed, dried and separated into a number of petri dishes for further experimentation. Another group of experiments involved a 5 min heat treatment at 100 C. A thermometer was placed in a 500 ml beaker which was then heated to 100 C with a Bunsen burner. The seeds were then placed in the beaker and the 100 C temperature was maintained for 5 min. After 5 min the seeds were placed in a petri dish at room temperature. The seeds were separated into various petri dishes for further experimentation. Several experiments also involved both HCl and heat treatments. The order of treatment was varied. In some of the experiments the HCl treatment was followed by the heat treatment and in other experiments the heat treatment was followed by an HCl treatment.

Seeds were stratified at 2 C for 14 to 90 days. Several experiments involved no stratification period as indicated by the 0 in Tables 1 and 2.

Identical germination preparations and experimental

Table 1. Summary of conditions imposed upon scarified seeds collected in the fall of 1982 from Bonayer Forest

Plate Number	Light or Dark	HCl Treatment	Heat Treatment	Days Stratified
1	Light	No	No	0
2	Dark	No	No	0
3	Light	No	No	14
4	Light	No	No	22
5	Light	No	No	30
6	Light	No	No	38
7	Light	No	No	45
8	Light	No	No	60
9	Light	No	No	90
10	Light	Yes	No	0
11	Dark	Yes	No	0
12	Light	Yes	No	21
13	Light	Yes	No	30
14	Light	Yes	No	45
15	Light	No	Yes	0
16	Dark	No	Yes	0
17	Light	No	Yes	21
18	Light	No	Yes	30
19	Light	No	Yes	45
20	Light	Yes	Yes	0
21	Dark	Yes	Yes	0
22	Light	Yes	Yes	21
23	Light	Yes	Yes	30
24	Light	Yes	Yes	45

Table 2. Summary of conditions imposed upon non-scarified seeds collected in the fall of 1982 from Bonayer Forest

Plate Number	Light or Dark	HCl Treatment	Heat Treatment	Days Stratified
1	Light	No	No	0
2	Dark	No	No	0
3	Light	No	No	14
4	Light	No	No	22
5	Light	No	No	30
6	Light	No	No	38
7	Light	No	No	45
8	Light	No	No	60
9	Light	No	No	90
10	Light	Yes	No	0
11	Dark	Yes	No	0
12	Light	Yes	No	21
13	Light	Yes	No	30
14	Light	Yes	No	45
15	Light	No	Yes	0
16	Dark	No	Yes	0
17	Light	No	Yes	21
18	Light	No	Yes	30
19	Light	No	Yes	45
20	Light	Yes	Yes	0
21	Dark	Yes	Yes	0
22	Light	Yes	Yes	21
23	Light	Yes	Yes	30
24	Light	Yes	Yes	45

conditions were conducted with the capsules and seeds collected in October, 1983. The seeds were immediately incorporated into the various experimental procedures eliminating the 3 month drying period observed for the 1982 seeds. The various experiments conducted are presented in Tables 3 and 4. In those experiments the arils were removed. Other experiments conducted with additional seeds included the aril.

GEOGRAPHIC VARIATION BY LEAF LENGTH AND WIDTH MEASUREMENTS

Data were collected from herbaria throughout the geographic range of *Euonymus americanus* as noted in Table 5. The lengths and widths of leaves from the second leaf pair were measured and averaged. Leaves from the third leaf pair were similarly measured and averaged. Leaf area estimates have been made by multiplying the length of the leaves by the width (Wargo, 1978; Pitcher, 1983). Similar leaf area estimates were made for the *E. americanus* leaves. The lengths and widths of the internodes between the second and third leaf pair were also measured.

The entire distribution of *E. americanus* can be subdivided into a number of regions based on several parameters. The nine regions indicated in Figure 1 are proposed based on the mean length of frost-free period and elevational differences. Another method used in delimiting these regions is based on a map of the potential natural vegetation of the United States (Küchler, 1964). The seven regions indicated in Figure 2

Table 3. Summary of conditions imposed upon scarified seeds collected in the fall of 1983 from Bonayer Forest

Plate Number	Light or Dark	HCl Treatment	Heat Treatment	Days Stratified	Aril Present
1	Light	No	No	0	No
2	Dark	No	No	0	No
3	Light	No	No	30	No
4	Light	No	No	60	No
5	Light	Yes	No	0	No
6	Dark	Yes	No	0	No
7	Light	Yes	No	30	No
8	Light	Yes	No	60	No
9	Light	No	Yes	0	No
10	Dark	No	Yes	0	No
11	Light	No	Yes	30	No
12	Light	No	Yes	60	No
13	Light	No	No	0	Yes
14	Dark	No	No	0	Yes
15	Light	No	No	30	Yes
16	Light	No	No	60	Yes

Table 4. Summary of conditions imposed upon non-scarified seeds collected in the fall of 1983 from Bonayer Forest

Plate Number	Light or Dark	HCl Treatment	Heat Treatment	Days Stratified	Aril Present
1	Light	No	No	0	No
2	Dark	No	No	0	No
3	Light	No	No	30	No
4	Light	No	No	60	No
5	Light	Yes	No	0	No
6	Dark	Yes	No	0	No
7	Light	Yes	No	30	No
8	Light	Yes	No	60	No
9	Light	No	Yes	0	No
10	Dark	No	Yes	0	No
11	Light	No	Yes	30	No
12	Light	No	Yes	60	No
13	Light	No	No	0	Yes
14	Dark	No	No	0	Yes
15	Light	No	No	30	Yes
16	Light	No	No	60	Yes

Table 5. A summary of the herbaria from which herbarium specimens of *Euonymus americanus* were provided for use in leaf and internode neasurements

HERBARIUM	LOCATION
National Herbarium	Washington, D.C.
North Carolina State University	Raleigh, NC
Ohio State University	Columbus, OH
Pennsylvania State University	University Park, PA
Southern Illinois University	Carbondale, IL
University of Alabama	University, AL
University of Florida	Gainesville, FL
University of Kentucky	Lexington, KY
University of Maryland	College Park, MD
University of Michigan	Ann Arbor, MI
University of Southwest Louisianna	Lafayette, LA
University of Tennessee	Knoxville, TN
Vanderbilt University	Nashville, TN
Virginia Polytechnic Institute and State University	Blacksburg, VA
West Virginia University	Morgantown, WV
Western Kentucky University	Bowling Green, KY

Figure 1. A partitioning of the distribution of *Euonymus americanus* into ten regions based on frost-free days and elevational differences.

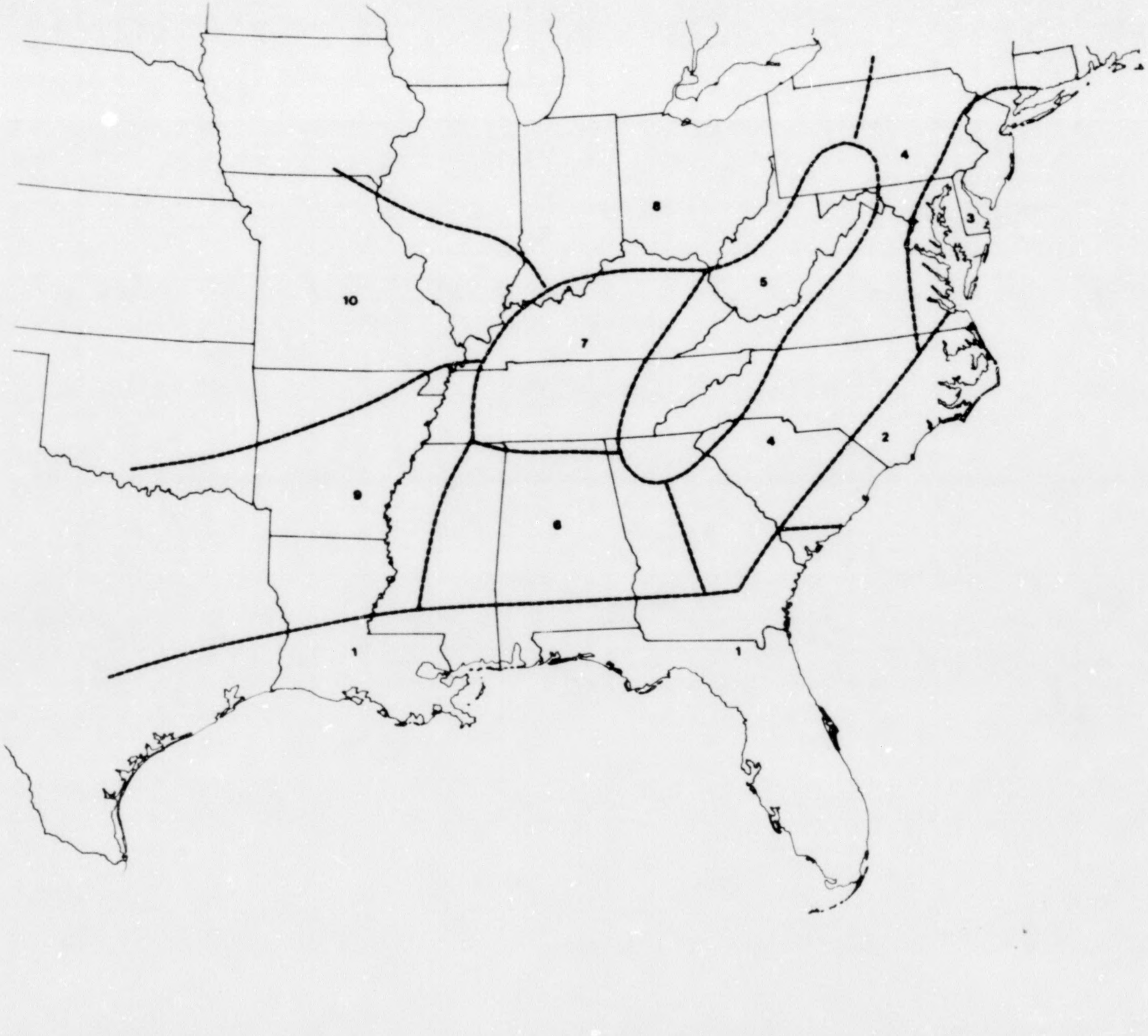
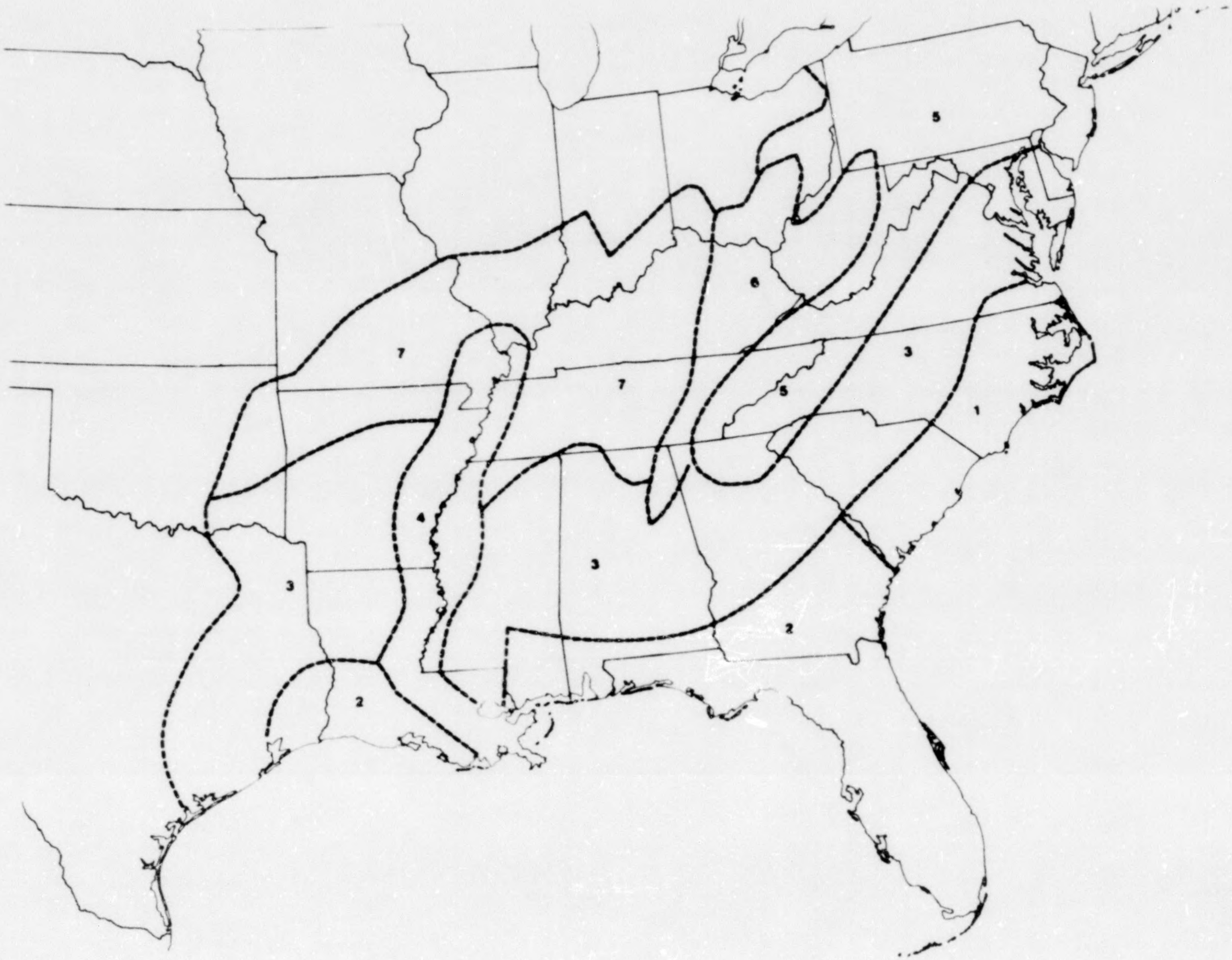

4
8
3
5
10
7
4
2
9
6
1
1

Figure 2. A partitioning of the distribution of *Euonymus americanus* into seven regions based on the Potential Natural Vegetation Map proposed by Küchler, 1964.


5
6
7
7
3
5
3
1
4
3
3
2
2

correspond to the major potential natural vegetation areas within the entire distribution of *E. americanus*. No specimens collected before March 30 were used in determining the second leaf pair measurements; however, the third leaf pair and internode measurements were made on specimens collected throughout the year. All measurements were made using herbarium specimens.

MEASUREMENTS OF SODIUM AND POTASSIUM IN THE LEAVES

Leaves from *Euonymus americanus*, *Corylus americana* Walt., *Asimina triloba* L., *Aralia spinosa* L. and *Lindera benzoin* L. were periodically collected from 6/13/83 to 10/19/83 at Bonayer Forest. The latter four species were included as a comparison to *E. americanus*.

The leaves were dried,and triplicate samples of 300 mg each were weighed for each plant species and placed in crucibles. The weight measurements were performed with a Mettler Type P163 balance. The leaf material was then dissolved with 3 ml of standard 12 M nitric acid. The digested material was then evaporated to dryness in a hood by placing the crucibles on a hot plate. Another 3 ml of standard 12 M nitric acid was added and again evaporated to dryness. The material was then washed in a 1:1 HCl to deionized water mixture and filtered through a #596 Schleicher and Schuell filter paper into a 100 ml volumetric flask. Additional deionized water was then filtered through to make a 100 ml solution.

A Coleman Model 51 Flame Photometer was used to analyze the prepared samples. Measurements of sodium and potassium levels were recorded. The low standard was a 2% Sterox solution, while the high standard was a 1.0 meq K/l and a 1.75 meq Na/l solution. The oxygen pressure was 13 atmospheres. Samples which were too concentrated were diluted by adding deionized water. Five ml of the original sample were diluted with five ml of deionized water and then analyzed.

RESULTS

Patterns of geographic variation and ion content in the leaves of *Euonymus americanus* were observed; seed germination experiments indicated a definite need for further investigation. Even though attempts were made to simulate natural environmental conditions, there was no observed seed germination in any of the experiments. The geographic variation of *E. americanus* did show some intriguing patterns from the data based on Figure 2. The data obtained through the use of Figure 1 did not show any significant geographic variations. The sodium and potassium content of the leaves of *E. americanus* through flame photometer analysis indicated a possible pattern over time; however, the ion content of the leaves of the other plants tested (*Corylus americana*, *Asimina triloba*, *Aralia spinosa* and *Lindera benzoin*) did not show any readily observable pattern.

GEOGRAPHIC VARIATION BY LEAF LENGTH AND WIDTH MEASUREMENTS

The data presented in Table 6 are summaries of the mean values for length, width, length X width and length/width of the second leaf pair, third leaf pair and internode between the two leaf pairs. The nine regions correspond to the regions indicated on Figure 1. The second leaf pair data from Table 6 indicate that the least leaf development occurred in regions 5 and 8, while the greatest development occurred in

Table 6. Mean values of length, width, length X width and length/width ratios for the second leaf pair, third leaf pair and internode between the two leaf pairs, based on Figure 1[1,2].

		Regions								
		1	2	3	4	5	6	7	8	9
Second leaf pair	L	5.47	5.79	5.80	5.49	5.05	5.37	5.39	4.99	5.40
	W	2.09	2.34	2.09	2.13	2.13	2.12	2.09	1.96	2.27
	LXW	11.43	13.55	12.12	11.69	10.76	11.38	11.27	9.78	12.26
	L/W	2.62	2.47	2.78	2.58	2.37	2.53	2.58	2.55	2.38
Third leaf pair	L	5.03	4.96	5.57	5.16	4.60	4.40	5.07	4.97	5.15
	W	2.04	2.08	2.07	2.10	1.90	1.84	2.07	2.11	2.14
	LXW	10.26	10.32	11.53	10.84	8.74	8.10	10.50	10.49	11.02
	L/W	2.46	2.38	2.69	2.46	2.42	2.39	2.45	2.36	2.41
Inter-node	L	3.75	4.15	4.16	4.15	3.47	3.81	3.49	3.11	3.96
	W	.12	.15	.14	.14	.13	.12	.13	.12	.13
	LXW	.45	.62	.58	.58	.45	.46	.45	.37	.51
	L/W	31.75	27.67	29.71	29.64	26.69	31.75	26.85	25.92	30.46

[1]Where L=length, W=width, LXW=length X width and L/W=length/width.

[2]All measurements were in centimeters.

region 2. The third leaf pair data from Table 6 indicate that the least leaf development occurred in region 6, while the greatest leaf development occurred in region 3. The internode data from Table 6 indicate that the least development occurred in region 8, while the greatest development occurred in regions 2 and 3.

Data shown in Table 7 are summaries of the mean values for length, width, length X width and length/width of the second leaf pair, third leaf pair and internode between the two leaf pairs. The seven regions in Table 7 correspond to the regions indicated on Figure 2. The second leaf pair data from Table 7 indicate that the least leaf development occurred in region 6, while the greatest development occurred in regions 1 and 4. The third leaf pair data from Table 7 indicate that the least leaf development occurred in region 6, while the greatest development occurred in region 1. The internode data from Table 7 indicate that the least leaf development occurred in region 5, while the greatest development occurred in region 1.

A statistical analysis of all the data was performed using a completely random, one-way analysis of variance test (Steel and Torrie, 1960). All values obtained for each region were used in this analysis. The resulting F values and significance at the 95% confidence level, based on Figure 1, are presented in Table 8. Significant differences between regions were observed only for the internode length and length

Table 7. Mean values of length, width, length X width and length/width ratios for the second leaf pair, third leaf pair and internode between the two leaf pairs, based on Figure 2[1,2].

		Regions						
		1	2	3	4	5	6	7
Second leaf pair	L	6.10	5.60	5.33	5.63	5.00	5.10	5.19
	W	2.42	1.99	2.07	2.53	2.02	1.94	2.04
	LXW	14.76	11.14	11.03	14.24	10.10	9.89	10.59
	L/W	2.52	2.81	2.57	2.23	2.48	2.63	2.54
Third leaf pair	L	5.68	5.12	4.81	5.05	4.66	4.61	5.22
	W	2.28	2.01	1.97	2.18	2.00	1.90	2.08
	LXW	12.95	10.29	9.48	11.01	9.32	8.76	10.86
	L/W	2.49	2.55	2.44	2.32	2.33	2.43	2.51
Inter-node	L	4.54	3.75	3.95	4.08	3.36	3.46	3.45
	W	.15	.12	.13	.13	.13	.13	.13
	LXW	.68	.45	.51	.53	.44	.45	.45
	L/W	30.27	31.25	30.38	31.38	25.85	26.62	26.54

[1]Where L=length, W=width, LXW=length X width and L/W=length/width.

[2]All measurements were in centimeters.

Table 8. Results of a completely random, one-way analysis of variance test performed on the length, width, length X width and length/width of the second leaf pair, third leaf pair and internode between the two leaf pairs, based on Figure 1[1].

		Treatment degrees of freedom	Error degrees of freedom	F value	Significant at .05 level
Second leaf pair	L	8	262	.76	No
	W	8	262	1.08	No
	LXW	8	260	1.02	No
	L/W	8	260	.67	No
Third leaf pair	L	8	208	1.60	No
	W	8	208	1.03	No
	LXW	8	208	1.70	No
	L/W	8	208	.89	No
Internode	L	8	296	2.42	Yes
	W	8	296	1.37	No
	LXW	8	296	1.87	Yes
	L/W	8	296	1.27	No

[1]Where L=length, W=width, LXW=length X width and L/W=length/width.

X width values. The second and third leaf pair values showed no significant differences between regions. Data obtained based on Figure 2 were also statistically analyzed by the one-way analysis of variance test. A summary of these values is shown in Table 9. In this analysis there was again a significant difference between regions for the internode length and length X width values. There were also significant differences between regions for the width and length X width values for the second leaf pair. The third leaf pair showed no significant differences between regions.

A second type of statistical analysis was performed on those values from Table 9 which showed significant differences between regions. Tukey's Multiple Range Test (Steel and Torrie, 1960) was used on the values for the width of the second leaf pair, the length X width of the second leaf pair and the length of the internode. Tukey's test was used for a comparison of the means of the regions to determine which regions were significantly different. The result of this test for the width values of the second leaf pair is shown in Table 10. Significant differences were found between regions 4 and 3,5,6 and 7. Region 4 corresponds to the Mississippi flood plain region. The result of Tukey's test for the length X width values of the second leaf pair is shown in Table 11. Significant differences were found between regions 6 and 1 and 6 and 4. The result of Tukey's test on the length of the internode showed no significant differences between the regions.

Table 9. Results of a completely random, one-way analysis of variance test performed on the length, width, length X width and length/width of the second leaf pair, third leaf pair and internode between the two leaf pairs, based on Figure 2[1].

		Treatment degrees of freedom	Error degrees of freedom	F value	Significant at .05 level
Second leaf pair	L	6	280	1.39	No
	W	6	280	3.54	Yes
	LXW	6	280	3.09	Yes
	L/W	6	280	1.15	No
Third leaf pair	L	6	210	1.65	No
	W	6	210	1.19	No
	LXW	6	210	2.08	No
	L/W	6	210	1.48	No
Inter-node	L	6	298	2.62	Yes
	W	6	298	1.68	No
	LXW	6	298	3.04	Yes
	L/W	6	298	1.95	No

[1]Where L=length, W=width, LXW=length X width and L/W=length/width.

Table 10. Results of Tukey's Multiple Range Test performed on the values obtained for the width of the second leaf pair, based on Figure 2[1].

		Regions						
		1	2	3	4	5	6	7
	1							
	2							
	3							
Regions	4			*		*	*	*
	5							
	6							
	7							

[1](*) Denotes significant differences at the .05 level.

Table 11. Results of Tukey's Multiple Range Test performed on the values obtained for the length X width of the second leaf pair, based on Figure 2[1].

		Regions						
		1	2	3	4	5	6	7
	1						*	
	2							
	3							
Regions	4						*	
	5							
	6							
	7							

[1] (*) Denotes significant differences at the .05 level.

MEASUREMENTS OF SODIUM AND POTASSIUM IN THE LEAVES

The results of the flame photometry analyses for potassium content are shown in Table 12. The values for the five plants tested over the time period from 6/13/83 to 10/19/83 are reported as mg/g dry weight. The calculated values do not seem to follow any simple pattern with the possible exception of _E. americanus_, which shows a general decline in potassium levels as the summer progressed. The results of the flame photometry analyses for sodium content are shown in Table 13. The values are reported in mg/g dry weight. The _E. americanus_ data indicate a gradual decline in sodium as the season progressed; the data obtained from the other plants show no pattern. Since _E. americanus_ is the principal plant under investigation, a graph of the potassium and sodium levels in the leaves of this plant is presented (Figure 3).

The reason for the lack of any pattern in the four other plants tested is probably due to non-random sampling. The leaves of these four plants were too large to permit more than one or two to be used in each sample; therefore a random distribution was not obtained. The leaves of _E. americanus_ are much smaller, and several were required for each sample. The data obtained from the _E. americanus_ leaves presumably had a more random distribution. The results for the four other plants are too inaccurate to permit any further discussion;however, the _E. americanus_ data are considered to be

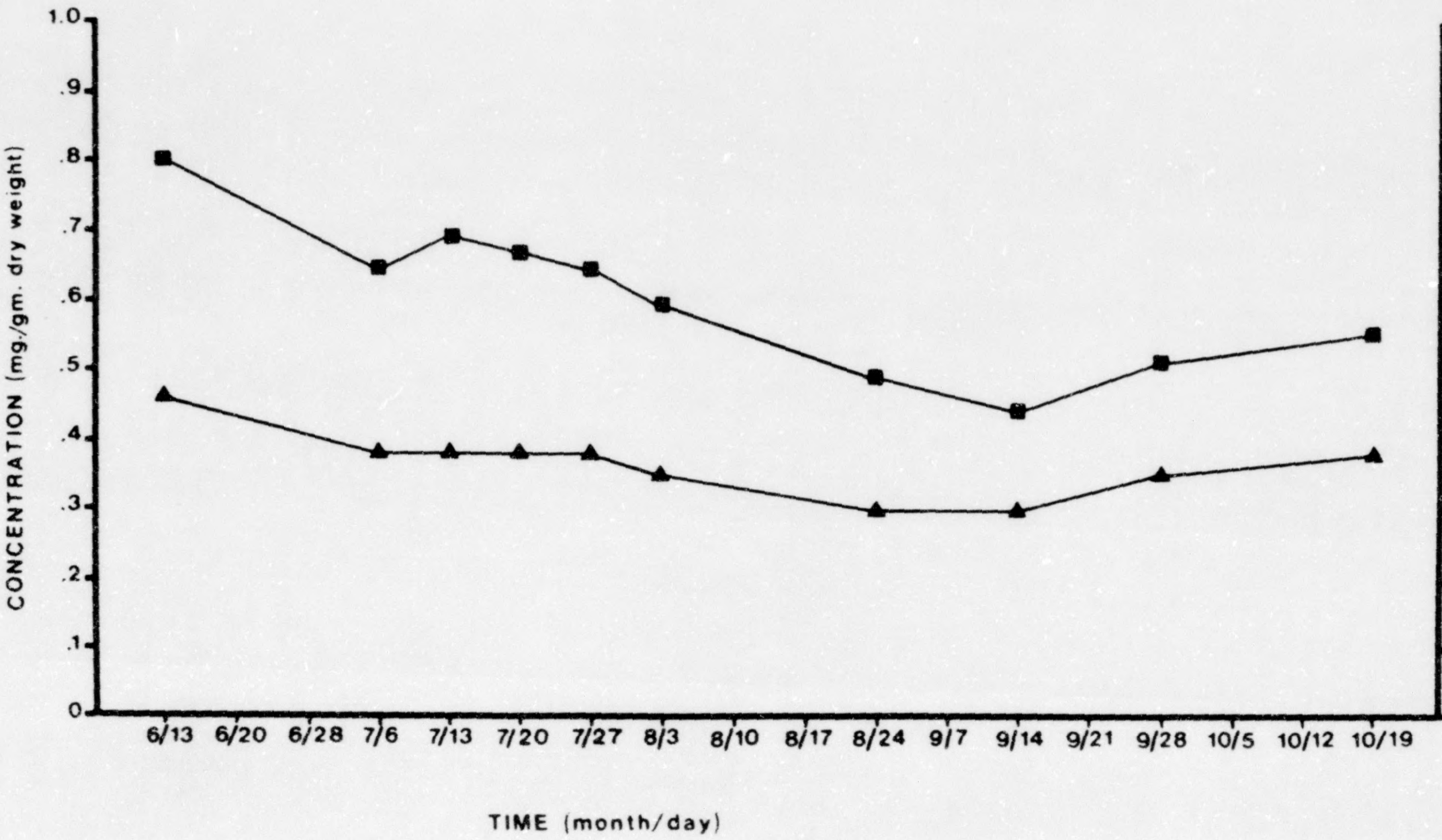
Table 12. Potassium content in the leaves of several species collected from Bonayer Forest over a four month period.

Date	Potassium content in mg/g dry weight				
	Euonymus americanus	*Corylus americana*	*Asimina triloba*	*Aralia spinosa*	*Lindera benzoin*
6/13	.80	.65	2.03	1.86	1.64
7/6	.63	.54	1.56	1.33	1.85
7/13	.69	.65	1.44	2.01	.89
7/20	.66	.71	1.85	1.76	1.48
7/27	.63	.71	1.31	1.22	1.10
8/3	.59	.35	1.55	1.49	1.89
8/24	.49	.57	1.72	2.22	1.31
9/14	.44	.45	1.62	.63	1.86
9/28	.51	.41	1.38	1.22	1.24
10/19	.56	.35	1.48	.54	1.06

Table 13. Sodium content in the leaves of several species collected from Bonayer Forest over a four month period.

Date	Sodium content in mg/g dry weight				
	Euonymus americanus	*Corylus americana*	*Asimina triloba*	*Aralia spinosa*	*Lindera benzoin*
6/13	.46	.34	.92	1.08	.56
7/6	.38	.31	.72	.69	.67
7/13	.38	.33	.65	1.17	.34
7/20	.38	.36	.87	.96	.56
7/27	.38	.37	.66	.66	.40
8/3	.35	.23	.40	.79	.67
8/24	.30	.33	.83	1.31	.49
9/14	.30	.31	.81	.39	.71
9/28	.36	.29	.67	.33	.46
10/19	.38	.26	.76	.35	.39

Figure 3. The concentrations of sodium (designated by darkened triangle) and potassium (designated by darkened square) in the leaves of *Euonymus americanus* collected from 6/13/83 to 10/19/83 at Bonayer Forest.


CONCENTRATION (mg./gm. dry weight)
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
6/13 6/20 6/28 7/6 7/13 7/20 7/27 8/3 8/10 8/17 8/24 9/7 9/14 9/21 9/28 10/5 10/12 10/19
TIME (month/day)

valid and will be discussed.

DISCUSSION

Phenotypic geographic variation from incompletely developed leaves and patterns of sodium and potassium content in the leaves over time were observed. Additionally, phenotypic geographic variation from fully developed leaves and preliminary seed germination experiments were unsuccessful. A review of the available literature pertaining to seed germination in the genus _Euonymus_ revealed that seeds did not germinate and embryos did not grow unless they had been exposed to a rather lengthy dormancy period. Attempts to reduce this dormancy period through several means in this investigation were unsuccessful. The expected geographic variation in fully developed leaves was not observed; however, incompletely developed leaves showed geographic variation due to the limitations of the growing season. The levels of sodium and potassium in the leaves showed a possible winter conditioning pattern through time, but a fall accumulation prior to leaf drop was not observed.

SEED GERMINATION EXPERIMENTS

Attempts to germinate the seeds of _Euonymus_ _americanus_ collected in the fall of 1982 were initiated only after they were allowed to "dry" for 3 months. Although all seeds remained under test conditions for at least 30 days, the original experiments, indicated by plates numbered 1-9 in Tables 1 and

2, met with failure. The results observed were unexpected, but a review of the literature provided several possible explanations.

The germination of seeds of several species of Euonymus has been found to be very poor (Nikolaeva, 1959). The seeds which germinated required moist soil conditions and a stratification period. Seeds of E. europaea L. were found to germinate after 6-7 months if they were also exposed to a 3 month warm stratification period (> 20 C). The seeds of E. planipes L., E. verrucosa L. and E. europaea were found to germinate after 5-18 months if they were kept at temperatures ranging from 0-10 C. In either case (warm or cold stratification), the total amount of time necessary to achieve germination exceeded 5 months. The seeds must undergo a period of dormancy.

Since this initial experiment, various researchers have attempted to reduce the time of dormancy through several means. Experiments have been focused on the germination of E. europaea seeds because this plant is widespread in Europe and Asia. The seeds of E. europaea can be induced to break dormancy early by gibberellic acid (Monin, 1967b), cold stratification and gibberellic acid (Monin, 1967d) and gibberellic acid and kinetin (Nikolaeva et al., 1973). Several detailed studies on the embryos of E. europaea showed development in the presence of gibberellic acid, especially gibberellic acid 3 (Beranger-Novat and Monin, 1971a, 1971b; Gambade, 1972;

Beranger-Novat et al., 1977). The effects of other hormones such as abscissic acid, beta-indole-acetic acid and kinetin on embryo development have also been tested. Apparently only gibberellic acid and, to some extent, kinetin were effective in reducing the length of dormancy (Nikolaeva et al., 1973; Nikolaeva et al., 1974). Although gibberellic acid has been found in the embryos (Monin, 1967b), an inhibitor of seed germination (trans-para coumaric acid) may also be present (Monin, 1967c). In addition, extracts from the arils of E. europaea have been found to inhibit protein synthesis (Gasperi-Campani et al., 1980). The presence of zeaxanthin and kaempferol in the arils (Hargreaves, 1971) may be important in inhibiting embryo development.

The main focus of the preceeding experiments was to find the combination of hormones and temperatures necessary for the germination of E. europaea seeds. The actual environmental conditions necessary for seed germination were not investigated and there is no published data on the germination of E. americanus seeds in particular. The remainder of the experiments (Tables 1 and 2) were attempts to simulate some possible environmental conditions to which E. americanus seeds might be exposed.

One of these environmental conditions is the scarification process. Scarification is usually an artificial means to allow oxygen and water to enter the seed. Often however, this process is effected in nature by birds (Ray et al., 1983). Seeds with a hard coat may undergo mechanical abrasion in the intestines of birds, whereby the seed coat becomes permeable to oxygen and water. If the deposition of these seeds is favorable, germination may occur. *Euonymus americanus* seeds are eaten by American robins (*Turdus migratorius*) and bluebirds (*Sialia sialis*) (Blakelock, 1951) and thereby may undergo scarification. An imitation of the scarification process (pricking the seeds with a dissecting needle) was performed in all the seed germination experiments reported in Table 1. Seeds in the germination experiments reported in Table 2 were not scarified and served as controls. Neither scarified nor non-scarified seeds were observed to germinate, but this does not mean that scarification is unimportant. A more thorough testing of the importance of scarification is needed. One way to achieve this would be to feed the seeds of *E. americanus* to various bird species and then observe the germination of those seeds after deposition.

The role of deer in the scarification process may also be important. Several reports have indicated that deer prefer various species of *Euonymus* over other plants as a food source (Halls and Alcaniz, 1972; Szmidt, 1975; Bobeck et al., 1979). The stems and leaves of *E. americanus* are one of the preferred foods of the white-tailed deer (*Odocoileus virginianus* L.) in Texas (Halls and Alcaniz, 1972) and the seeds may undergo scarification while in the digestive tract of these deer. The process may be similar to that found in black bear. The black bear often eats the entire fruit of many plants, but the seeds remain undigested and pass through the intestinal tract. A significant difference has been found in the percent germination and germination rate between those seeds which pass through the digestive tract and those which remain exposed to the outside environment. The seeds of several different plants which have passed through the digestive tract often show the greater germination rate as well as percent germination (Rogers and Applegate, 1983). The actual suspected cause of the greater germination rates may be either mechanical or acid scarification. Either or both of these processes may be important in the possible scarification of E. americanus seeds resulting from the food preferences of deer.

Scarification due to acids present in the intestines of herbivorous mammals was only part of the reason for seed germination experiments involving a 3% HCl solution. Another environmental factor to consider is the possibility that tannic acid may function in "softening" the seed coat of E. americanus seeds. The seeds used in this study were all collected from a relict hardwood forest which contained mature oak trees of various species (Bougher and Winstead, 1974). The leaves of oak trees contain tannic acid (Ting, 1983) which may function in breaking down the seed coat of E. americanus seeds. An initial attempt to test the possibility of any acid scarification is indicated by plates numbered 10-14 in Tables 1 and 2. The results showed no seed germination. A more thorough investigation pertaining to acid scarification of E. americanus seeds is necessary.

Another possible factor involved in the seed germination of some plants is the necessity for fire. The seeds of a typical chaparral plant, Ceanothus megacarpus Nutt., are stimulated to undergo germination by temperatures of 100 C for 5 min (Hadley, 1961). Although the geographic range of E. americanus does not include the typical vegetation which requires fire as a necessary part of the reproductive cycle, several seed germination experiments which involved a similar heating of the seeds were undertaken (see plates numbered 15-19 in Tables 1 and 2). Again, there was no observed seed germination. The limited scope of these experiments was not a particularly valid test of any heat requirement. There is probably no extreme heat requirement for the germination of these seeds; however, there may be a warm stratification requirement similar to that reported for E. europaea seeds Nikolaeva, 1959).

A cold stratification period might also be necessary for E. americanus seeds. Many plants, especially ornamentals, require a period of cold stratification before germination may begin (Ray et al., 1983). All of the experiments in Tables 1 and 2 had varying numbers of days during which the seeds were kept at a constant temperature of 2 C in the dark; however, these seeds did not germinate. The length of stratification of these seeds might not have been sufficient, since E. europaea seeds often require at least 3 months of cold before they will germinate (Nikolaeva, 1959). A more thorough investigation of the stratification requirement is needed.

The remainder of the experiments in Tables 1 and 2 (plates numbered 20-24) were various combinations of the above mentioned treatments, but the results were once again disappointing.

Another series of experiments was performed with the seeds collected in the fall of 1983. Whereas the seeds collected in the fall of 1982 were exposed to a 3 month "drying" period, the seeds collected in the fall of 1983 were immediately incorporated into germination experiments. The 1983 germination experiments were similar to those performed on the 1982 seeds (see Tables 3 and 4). As with the 1982 germination experiments, no seed germination was observed.

Although disappointing, the results do not indicate a "dead end" to _E. americanus_ seed germination experiments. In fact, more seed germination experimentation is needed. The germination of _E. americanus_ seeds is evidently not a simple process and probably involves a specific pattern of requirements. The processes of stratification (warm and/or cold) and possibly scarification (mechanical and/or chemical) may play an essential role in this specific pattern.

GEOGRAPHIC VARIATION BY LEAF LENGTH AND WIDTH MEASUREMENTS

A second phase of experimentation conducted on _E. americanus_ was an investigation of phenotypic differences in the leaves. Differences in the length and width of the leaves were expected within the entire range of distribution. Similar investigations with several different plants have shown phenotypic and sometimes genotypic geographic variation with respect to photoperiod adaptation in Xanthium strumarium L. (Ray and Alexander, 1966), leaf drop in Liquidambar styraciflua L. (McMillan and Winstead, 1976) and chlorophyll levels in Xanthium strumarium (Abdulrahman and Winstead, 1977). An investigation of geographic variation in the leaves of black cherry (Prunus serotina Ehrh.) revealed that northern populations have longer but more narrow leaves than southern populations (Pitcher, 1983). The leaf area of black cherry also shows a pattern of geographic variation (Pitcher, 1983).

In order to test for phenotypic differences in the leaves, the second and third leaf pair were chosen because they were expected to have reached maximum development within the growing season. Any differences would then be due to factors other than the length of the growing season. In addition to the second and third leaf pair, measurements were also taken for the internode between the two leaf pairs. The results presented in Table 6 show no significant differences as indicated by the analysis of variance test summarized in Table 8. Therefore, the nine regions indicated on Figure 1 showed no phenotypic differences in the values for the length, width, length X width and length/width of the second leaf pair, third leaf pair and internode.

In order to be certain that there was no geographic variation, a second map based on the potential vegetation within the geographic range of E. americanus was proposed.

The results of an analysis of variance test presented in Table 7 show some significant differences (see Table 9). The significant differences noted in the values for the width and length X width of the second leaf pair should probably be attributed to the length of the growing season. This reasoning is based on the observation that no significant differences were noted for any measurements involving the third leaf pair. Since the third leaf pair actually developed before the second leaf pair, it must have reached maximum development within the growing season.

Regional differences based on Figure 2 were noted for the values of the second leaf pair (see Table 10). Regions 1, 2 and 4 were not significantly different. The first two are coastal regions and are probably influenced by more constant environmental conditions. Region 4 includes the Mississippi valley, which also has fewer climatic fluctuations than do more inland areas. Regions 1, 2, 3, 5, 6 and 7 were also not significantly different, thereby supporting the idea of a lack of "real" phenotypic variation in the leaves of this plant. Regions 4 is significantly different from regions 3, 5, 6 and 7. The reason may be the longer growing season present in the Mississippi valley.

The regions among which significant differences were found for length X width values of the second leaf pair may be seen in Table 11. Significant differences were found only between regions 6 and 1 and 4. The significant difference between regions 6 and 4 partially coincides with the differences found between regions for the width of the second leaf pair. The leaf area of the second leaf pair may therefore also be affected by the length of the growing season. The difference between regions 6 and 1 was similarly presumed to be due to differences in the length of the growing season.

Significant differences were also found in the values obtained for the length and length X width of the internode. Tukey's Multiple Range Test showed no significant regional differences with respect to length. The measurements of the width of the internode proved to be an invalid parameter because the width often depended on the extent to which the herbarium specimens had been pressed. Tukey's Multiple Range Test was therefore not applied to the length X width values from the internode measurements.

Two important points should be remembered from these experiments. First, phenotypic differences in the fully developed leaves of the third leaf pair of E. americanus were not observed. In contrast, fully developed leaves from other plants may show geographic variation (Pitcher, 1983). Second, significant phenotypic differences were noted for the incompletely developed leaves of the second leaf pair. In order to determine the extent to which climate affects development, measurements for the first leaf pair should be made. Since the first leaf pair is actually the last to develop, any consistent differences over the entire sampling period (1875-1983) would probably be due to differences in the length of the growing season.

The possibility also remains that there are genotypic differences in the leaves of this plant. In order to test for this possibility, standardized light, temperature and moisture conditions would have to be set up. Living specimens or seeds collected from the different regions could then be grown under these standardized conditions. Any observed variation would then be caused by genotypic differences.

MEASUREMENTS OF SODIUM AND POTASSIUM IN THE LEAVES

Euonymus americanus is a very common understory inhabitant of relict hardwood forests (Bougher and Winstead, 1974). In fact, the frequency of its occurrence ($>$ 98%) is so high that it may be a critical component in these forests. It may play a "role" within the forest ecosystem similar to that of flowering dogwood (*Cornus florida* L.), which acts as a calcium pump (Thomas, 1969). Calcium is taken up from the soil through the roots of the dogwood and collected in the leaves. In the fall when leaf drop occurs, the stored calcium in the leaves is released through degradation. In a similar manner, some ion(s) may accumulate in the leaves of *E. americanus*, only to be released in the fall when degradation of the fallen leaves occurs. A preliminary investigation for such an ion(s) was performed by collecting the leaves of several plants growing in a relict hardwood forest. The levels of two ions, sodium and potassium, were examined in leaves collected over a four month period. With the exception of *C. americana*, the levels of these ions were higher in the accessory species: *A. triloba*, *A. spinosa* and *L. benzoin*. These results indicate that *E. americanus* does not have a significant role in the cycling of these two elements. Both the sodium and potassium levels in the leaves of *E. americanus* declined from June 13th to September 14th. During this period of the life cycle of *E. americanus*, the development of the sexual reproductive stage takes place. By mid to late September the capsules containing the seeds are dropped from the plant. The declines observed for the sodium and potassium levels may indicate that these ions are translocated from the leaves and into the developing capsules and seeds. When the capsules and seeds are dropped from the plant, the levels of sodium and potassium may once again increase in the leaves. The problem with this theory is that although potassium can be translocated, sodium cannot (Ting, 1982).

A second explanation for the gradual decline in the levels of sodium and potassium over the first three months, with an increase in the last month, is a physiological conditioning of the plant in preparation for winter. Since the leaves of *E. americanus* are deciduous, excess sodium and potassium could be accumulated in the leaves to be shed, thereby resulting in the "correct" winter levels of these ions.

Both of the preceeding explanations would require additional research to determine the extent to which either might occur. In addition, other ions should be tested to determine if there is any one which might show a cyclical pattern. It should be noted that the sodium levels were fairly high in this four month study. Since sodium is not one of the eight macroelements

(Ting, 1982), the levels are surprising. The possibility exists that the plant is a halophyte and merely tolerates high levels of sodium in the soil. A soil analysis of the collection site was not performed, therefore no correlations can be drawn. A less likely possibility is that E. americanus is a C_4 plant, since all C_4 plants require sodium for the carbon reduction stage of photosynthesis (Ting, 1982). Further research concerning this subject is necessary.

SUMMARY

Euonymus americanus is something of an ecological enigma. The extremely high frequency of its occurrence in a relict hardwood forest is surprising, but even more surprising is the fact that there is no published autecological research concerning this plant. This project was an initial autecological investigation of *E. americanus* in a relict hardwood forest in south-central Kentucky and an examination of the extent of leaf phenotypic variation within the total range of distribution.

The results of an investigation quantifying the sodium and potassium levels in the leaves of *E. americanus* collected from a relict hardwood forest during a four month period showed a possible winter conditioning pattern. The high sodium levels observed in this experiment may have been due to the possibility that *E. americanus* is a C_4 plant. A further investigation of this possibility, along with an examination of the levels of other elements in the leaves, is necessary. Generalized seed germination experiments resulted in no seed germination, but more in-depth investigations are needed. The most likely possibility seems to be that the germination of *E. americanus* seeds requires a specific sequence of environmental conditioning. The limitations of different growing seasons in the distribution of this plant probably determined the observed differences in the leaves. Leaves which were fully

developed showed no statistically significant patterns of phenotypic variation.

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